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Review

Timing in plants – A rhythmic arrangement

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ABSTRACT

The circadian clock regulates many aspects of plant physiology, growth and development. It produces daily rhythms of growth and metabolism, and interacts with signalling pathways controlling environmental responses over the course of a day or a year. Over the last decade, a combination of empirical research in molecular genetics and mathematical modelling, mostly utilising *Arabidopsis thaliana*, has led to the identification of many plant clock components and an understanding of their interlocking roles within the biochemical mechanism. The plant clock shares many characteristics of circadian clocks in other taxa, being temperature-compensated, capable of generating endogenous rhythms, of entraining to environmental cycles and regulated by means of transcription–translation feedback loops; however, few, if any, components of the plant clock appear to be shared with other organisms, indicating an independent evolutionary origin. In this review, we describe our current understanding of the central clockwork and how it receives input and regulates outputs. We also discuss the interaction between the clock and the environment, identifying areas, such as the integration of non-photic stimuli, where future work will lead to a fuller understanding of how the circadian system is embedded in plant physiology.

1. Introduction: necessity and purpose of clocks

Always and everywhere there is change. The planet spins on its axis and this rotation gives rise to the pattern of day and night. The tilting of the planet in its orbit tips one half or the other towards the sun and thus the seasons wax and wane across the year. Save for those organisms that live in the deeps of the earth or in the depths of the sea, all living things experience these changes and thus face the challenge of coordinating their lives with the Earth's rotation. A common response has been the evolution of an endogenous clock that can be set by the rising or the setting sun. Such biological clocks are exactly analogous to our mechanical clocks and watches that, before the invention of the quartz mechanism, needed to be reset each day to keep them aligned with national time (the BBC still broadcasts the time signal "the pips" each hour for this purpose). In the absence of such coordination, clocks in different places are likely to drift with respect to each other. This is of no matter in and of itself but becomes important in a network – it was the advent of railways, and more particularly railway timetables that caused the United Kingdom to adopt a single, central time [1]; until this time towns and villages would set clocks based upon the local sunrise. This analogy nicely makes two important points about biological clocks, firstly that they produce patterns of rhythmicity with a period close to that of a solar day and secondly that they are entrainable to environmental rhythms of light and darkness. Moreover, a multicellular organism often contains more than one clock. These may be independent from each other, taking their timecues from the environment, or else arranged in a hierarchy with one masterclock setting time for the others.

The study of circadian clocks in any organism can be considered from two, complementary viewpoints: the clock's mechanism and the clock's purpose. The purpose has been touched upon, the mechanism is being gradually elucidated. It is helpful to consider the circadian system as consisting of three interconnected pieces (Fig. 1): the receptors that perceive the environmental signals and provide input, the core oscillator that generates the rhythms, and the outputs ('hands') that produce rhythmic physiology and behaviour.

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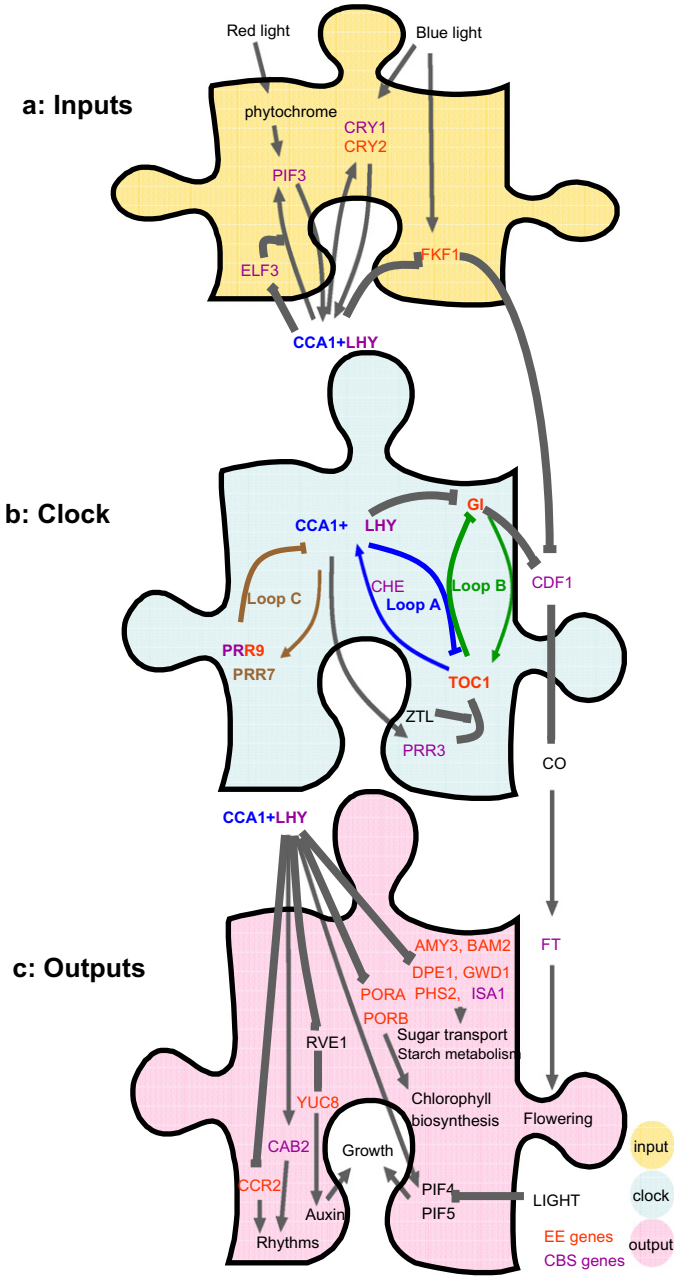

Fig. 1. Summary of the plant circadian system showing how the clock regulates aspects of plant growth and life history. The circadian system is visualised as three, interconnected pieces corresponding to inputs, oscillator and outputs. Many of the genes and proteins shown in this figure display rhythmic expression although this may vary with the zeitgeber; readers wishing for further information on this are referred to the study of Michael et al. '08 [58]. (a) Light input to the clock. Light is perceived via phytochromes and cryptochromes, the two major classes of photoreceptor. Feedback from the clock ensures that inputs are gated and so the intensity of a given stimulus varies through the day. (b) The three loop model of the plant clock. The clock loops are a simplified version of those described by empirical research and predictive models [6,7,106] and not all elements are included. Core clock components are printed in bold font. (c) Output pathways of the clock. Genes containing CCA1 binding sites or evening elements in their promoters are printed in purple or red, respectively (*PRR9* contains both). Full versions of gene names are given in the text or references therein.

2. Clock mechanism and sensory inputs

2.1. Transcription–translation feedback loops

Circadian clock mechanisms have been well characterised from animal, fungal and plant systems (in this issue [2–4]). At the core of each clockwork is an autoregulatory feedback loop whereby proteins regulate their own transcription, either directly or indirectly. Such a system has been described for *Arabidopsis* whereby the core clock consists of three tightly interlocked feedback loops [5–7] (Fig. 1b).

The circadian clock is typically reset by daily changes in light and temperature. In plants such as *Arabidopsis thaliana*, entraining light signals are perceived through a range of photoreceptors including the phytochromes, which receive far-red and red light wavelengths, and cryptochromes, sensitive to blue light [8,9]; plant clocks are also entrained by daily temperature rhythms but the perception and transduction of such signals is not fully understood. However, a central function of the circadian clock is to sustain robust cycling across a wide range of light and temperature conditions, and the plant circadian system is well buffered across a range of temperatures by a compensatory mechanism [10].

The first loop to be characterized in *Arabidopsis* comprised two negative elements, the transcription factors CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), which both repress expression of the positive factor TIMING OF CAB EXPRESSION 1 (TOC1), a member of the PSEUDORESPONSE REGULATOR (PRR) protein family [11,12] (whose members are expressed rhythmically, with consecutive peaks about 2–3 h apart, over the day (or subjective day) beginning with *PRR9* in the morning and ending with *TOC1/PRR1* in the evening [13–15]). Levels of CCA1 and LHY RNA and proteins peak at the beginning of the day (or subjective day) and those of TOC1 do so in the evening (or subjective evening). During the day, CCA1 and LHY are phosphorylated by casein kinases and the modified proteins subsequently degraded. Their disappearance leads to de-repression of *TOC1* expression in the afternoon (reviewed by [16]). TOC1 then acts to promote expression of *CCA1* and *LHY*; in the case of *CCA1*, TOC1 forms a complex with a class1 TCP transcription factor CCA1 HIKING EXPEDITION (CHE) which binds the *CCA1* promoter [17] and thus closes the loop. However, the TOC1–CHE complex does not bind the LHY promoter thus other, so-far unknown factors must play a similar role in regulating expression of this gene.

CCA1 and LHY interact as positive elements in a second loop in which the PRRs (specifically, PRR7 and PRR9) feedback to repress expression of CCA1 and LHY [18–22]. The third loop again involves TOC1, this time as a negative regulator of *GIGANTEA* (*GI*), which in turn appears to increase *TOC1* transcription [6]. GI has been implicated in control of flowering and circadian clock function, where low levels of GI expression prolonged circadian period and delayed flowering [23,24]. The robustness of the clock system is revealed by the persistence of rhythmicity in single knockouts of any of these genes; many double knockouts are also rhythmic, albeit with altered period and phase, although the *cca1;lhy;toc1* or *prr5;prr7;prr9* triple mutants are arrhythmic [25,26]. A similarly robust network is observed in clocks of other organisms (see, for example, [27]) and this is almost certainly a required property to buffer the system against both loss of a single component and environmental noise.

These loops of temporally-coordinated gene expression are synchronised by post-translational modification of their protein products. TOC1 protein is regulated by the F-Box protein ZEITLUPE (ZTL) which targets it for degradation via the proteasome by a CULLIN1-containing Skp1-Cullin-F-box protein (SCF) complex at night [28]. *ztl* mutations increase the free-running period of the circadian clock; ZTL protein is rhythmic with a night time peak but levels of *ZTL* transcript are constant over 24 h. GI, also expressed during the evening and night, associates with ZTL in a light-dependent manner and shapes its expression post-translationally. Interaction with ZTL also leads to degradation by the proteasome of PRR5, another member of the TOC1/PRR family [29]. The access of ZTL to its substrates TOC1 and PRR5 is regulated by multiple factors

including PRR3, GI and (blue) light [29–32]. ZTL thus plays a central role in regulating the resetting of the clock in response to dusk; however, all the evidence to date is that the protein acts post-translationally to determine protein stability and turnover.

Control of target genes regulated by CCA1 and LHY is via *cis*-elements such as the evening element (EE) [33] or the CCA1-binding site (CBS) [34,35] present in their promoters. The EE (AAAATATCT) was identified as a motif found in a number of genes under circadian regulation that exhibited peak expression at the end of the day [33]. The EE in the *TOC1* promoter is bound by CCA1 [12] and LHY [36].

2.2. Light input to the plant clock

The photoreceptors responsible for clock resetting have been well characterised. The red-absorbing phytochromes and the blue-absorbing cryptochromes both play a major role, covering the extremes of the visible spectrum. Evidence of their involvement comes from the fact that the free-running period length of the clock lengthens in their absence. Jurgen Aschoff observed that the free-running period length of the clock is very closely tied to light intensity. As light intensity decreases the period length of the rhythm lengthens in diurnal organisms and shortens in nocturnal organisms, presumed to be the net effect of decreased input to the clock by the resetting photoreceptors; this is known as *Aschoff's rule* [37]. Similarly, absence of the specific phytochromes causes period lengthening in red light, while absence of cryptochromes causes period lengthening in blue light [38–40]. The light labile phytochrome A perceives low fluence rates of red light while the light stable phytochromes B, D and E perceive higher fluence rates of red light. Equally, the light labile cryptochrome 2 perceives low fluence rates of blue light while the light stable cryptochrome 1 perceive higher fluence rates of blue light. Thus, the plant is responsive to the full range of light conditions in which it may find itself.

The mechanism of action of the phytochromes and cryptochromes on the clock is not fully understood. Expression of the morning loop genes *CCA1* and *LHY* is responsive to phytochrome input [34,41] (Fig. 1a). Both contain classical light-responsive G-box elements (CACGTG) within their promoters. In both cases this G-box has been shown to be the target for the PHYTOCHROME-INTERACTING 3 PIF3 transcriptional repressor [41]. PIF3 is found bound to G-boxes in dark grown seedlings and binding of photoactive phytochrome results in the removal and degradation of PIF3, promoting transcription of target genes [42]. However, studies of PIF3 overexpressors and loss of function mutants have been unable to demonstrate a role for PIF3 in the clock. Such mutants in PIF3 show normal circadian gene expression and also show normal clock resetting in response to red light pulses given following transfer of seedlings to darkness [43]. *CCA1* and *LHY* as well as *TOC1* and *GI*, like many clock associated genes have been demonstrated to be targets for the ELONGATED HYPOCOTYL 5 (HY5) transcription factor which plays a major role in light signalling downstream of both phytochromes and cryptochromes. However, expression levels of CCA1, LHY, TOC1 and GI were not significantly altered in the hy5 mutant [44]. It is possible that there is a redundancy in the action of multiple *cis* and/or *trans* acting-factors in regulation of light input to the clock.

The one case where a direct link has been demonstrated between a photoreceptor and a central clock component is that of the clock-specific blue light photoreceptor, ZEITLUPE (ZTL). ZTL is a flavin-binding F-box protein capable of absorption of blue light. In response to blue light, ZTL protein binds to the GI protein and as a result ZTL protein is stabilised [30]. ZTL also directly interacts with TOC1 protein and mediates dark-dependent degradation of the TOC1 via the proteasome pathway [45]. ZTL therefore accumulates maximally just prior to dusk and, following dusk, it brings about a rapid decrease in TOC1 protein. Such an effect would provide a strong dusk signal. Consistent with this role in mediating TOC1 degradation, the *ztl* mutant displays a long period length in constant light [46].

Despite the limited understanding of the mechanisms involved in clock resetting by light, the fact that there is, none-the-less, a very close link between light signalling and the plant clock is perhaps best emphasised by the fact the majority of central clock components are light regulated. Plants are particularly tied to the light environment by the process of photosynthesis and this perhaps has increased the complexity of the plant clock.

2.3. Gating of light inputs

The specific sensitivity of the clock to dawn and dusk signals is achieved by a phenomenon known as gating. Here, a metaphorical gate closes to prevent light from causing resetting at inappropriate times. This is particularly important during the middle part of day when light is present but does not confer any information about the time. If the clock were responsive to light at this time there would be a constant process of resetting occurring preventing the clock from progressing. The phenomenon of gating should not be confused with that of photoadaptation whereby, following an initial acute response to the incidence of light, further responses are down-regulated as the duration of light incidence continues (see [3] in this issue for a full discussion of photoadaptation with respect to *Neurospora crassa*). In gating of light inputs all light responses are moderated. The mechanism by which this daytime period of insensitivity to light is achieved is not known. One aspect contributing to the variation in sensitivity may be an oscillation in the levels of photoreceptor proteins. Phytochromes A, B, D and E show a circadian rhythm of transcription and all phytochromes show rhythmic transcription in light:dark cycles. The exact timing of the peaks varies between the phy species: in light:dark cycles, *PHYA* shows two peaks in transcription, one just after dawn and another shortly before dusk; *PHYB*, *PHYD* and *PHYE* show a peak of transcription during the morning; and *PHYC* shows a peak of transcription shortly before dusk [47]. Similarly, the cryptochromes show rhythmic transcription: in light/dark cycles, *CRY1* shows a peak in transcription during the morning; while *CRY2* shows a peak of transcription shortly before dusk [47]. Such an oscillation in photoreceptor levels with peaks around dawn and dusk fits perfectly with the heightened sensitivity of the clock to light for resetting at these times. However, it has not proved possible to detect such diurnal changes at the level of protein. It remains possible that newly-synthesised photoreceptor could behave differently from existing photoreceptor but, to date, a mechanistic link between phy or cry photoreceptor levels and gating has not been demonstrated.

ZTL, in contrast, is constitutively expressed at transcriptional level but clock regulated at the post-translational level. As a result of the stabilisation of ZTL by GI, ZTL is only present at high levels when GI is present. The rhythmic transcription of GI, therefore, drives a rhythm in ZTL protein levels. As a consequence, the light-dependent effect of ZTL on TOC1 levels is effectively gated and this forms a definitive example of the gating of light input through regulation of photoreceptor levels [30].

A number of proteins acting upon the transduction of photoreceptor signals have also been demonstrated to play a role in gating. EARLY FLOWERING 3 (ELF3) is essential to allow the clock to continue running beyond subjective dusk as the clock in the *elf3* mutant stops at this point if plants are transferred to constant light [48]. It is proposed that, in the absence of ELF3 to gate light input, the clock is continually reset until the plants are returned to darkness. Thus ELF3 might be predicted to be very important in

maintaining the rhythm as days are lengthening towards summer and, indeed, *elf3* mutants show an inability to adapt properly to long days, becoming responsive rather than predictive [48–50]. However, although clearly essential for normal circadian function, this mechanism is clearly distinct from the daytime reduction in sensitivity to light and absence of clock resetting during the day.

FHY3 has been proposed to act in daytime gating but appears to be red-light specific. The *fhy3* mutant becomes arrhythmic in constant red light and shows a loss of gating of clock resetting responses to red light during the subjective daytime [51]. FHY3 was more recently shown to act as a transcription factor along with its close homologue, FAR1, binding to an FBS in the promoter of FHY1, a component of the phyA signal transduction pathway [52]. Although the role of FHY3 in phyA signalling is clearly distinct from its role in light input to the clock, it is, none-the-less, possible that the mechanism of action of FHY3 in the clock may be direct regulation of light response of clock genes.

TIME FOR COFFEE (TIC) is another component that has been demonstrated to be involved in gating. As in *elf3*, the clock stops in *tic* mutants following transfer to constant light. In the case of the *tic* mutant the clock stops after 19 h in constant light indicating that TIC acts in the mid to late night to allow rhythmicity to be maintained. This duration of light is not something that would be encountered in nature but the proposed role for TIC in gating of clock resetting is confirmed by the failure of *tic* mutants to show anticipation of light/dark transitions in long days. Instead the *tic* mutant shows a more responsive phenotype similar to that seen in *elf3* mutants [53]. TIC was shown to encode a novel nuclear protein showing constitutive expression [54]. Its mechanism of action is not suggested by this finding but, curiously, TIC was recently demonstrated to be involved in iron homeostasis [55]. Whether this will prove to be related to its role in maintenance of clock function remains to be determined.

The *elf4* mutant also shows defects in circadian gating. Beyond dusk on the second day following transfer to darkness, *elf4* seedlings are unable to moderate the resetting effect a short pulse of light. Even a 5 min pulse of red light is sufficient to cause resetting of the clock in *elf4* seedlings whilst in wild type seedlings the rhythm continues unaffected [56]. Such a defect suggests a role for ELF4 around dusk in wild type seedlings. As with other gating mutants, *elf4* also fails properly to adjust to variation in daylength, being unable to anticipate the timing of dawn and dusk under such conditions. ELF4 has been shown to play an important role in the light induction of *CCA1* expression but its exact mechanism is also unknown [57].

3. Clock control of outputs

3.1. Regulatory motifs

The clock controls many aspects of plant physiology (Fig. 1c). Up to 89% of *Arabidopsis* genes show rhythmic expression under one set of experimental conditions or another [58]. Some rhythms are driven directly by environmental cycles of light or temperature but that others remain in a constant environment implies they are directly coupled to the clock itself. In many cases timing of expression of a transcript will differ between entrained and free-running conditions or between entrainment to temperature and light cycles, showing that the final phenotype results from an interaction between the clock and the response to the environment [58]. Peak expression of clock controlled genes occurs at all times of subjective day and night thus mechanisms have evolved to set the phase of a rhythm as well as its period. Given that the majority of *Arabidopsis* genes are at least conditionally rhythmic much attention has focused on transcriptional regulation by the clock.

Two of the first proteins identified with the circadian clock were the transcription factors CCA1 and LHY. Transcription of both is light regulated via interactions with PHYTOCHROME INTERACTING FACTOR 3 (PIF3), at least in aetiolated seedlings [41]. CCA1 was originally demonstrated to bind a motif (AAAAATCT), thus called the CCA1 binding site (CBS), in genes showing peak expression in the middle of the day [59]. Subsequently it was found that both proteins bound a closely related motif, the evening element (EE; AAATATCT) [33]. The EE is in itself sufficient to confer an evening phase of gene expression [60]; it has been suggested that the CBS confers morning phase [61], however additional flanking sequences may be required for this, as multimerising the core CBS-sequence produced evening-phased rhythms [60]. The modifying effects of flanking sequence are shown clearly by using *PRR9* as an example; this gene is normally expressed in the (subjective) morning and its promoter contains copies of both the EE and the CBS. Normally, mutating the EE abolishes rhythmic gene expression; however, in the case of *PRR9,* mutation of the EE plus retention of the flanking regions altered expression to a morning phase. Analysis of this region found a sequence AACCAC almost immediately upstream of the EE was required for this; this motif was named the morning element (ME) [60]. Its sequence overlaps with that of a motif (CACT**AACCAC**) overrepresented in the promoters of light regulated genes that respond to phytochrome A [62].

The binding of CCA1 to the EE in the *TOC1* promoter is determined by the chromatin structure. CCA1 opposes H3 acetylation to regulate levels of *TOC1* transcript. Induction of *TOC1* is dependent on circadian cycles of histone deacetylation. In the evening, histone deacetylases cause the chromatin to condense and become inaccessible to CCA1 whereas in the morning histone acetylation allows CCA1 binding and hence repression of *TOC1* transcription [12]. Whilst this mechanism has been directly demonstrated only for CCA1 acting on the *TOC1* promoter it is highly probable that the same process regulates other genes regulated by CCA1 and LHY. Chromatin remodelling also regulates circadian gene expression in other clockworks including that of mammals [63].

3.2. Regulation of growth

The clock also regulates the timing of plant growth (Fig. 1c). In constant light, hypocotyl elongation occurs at the end of the subjective day [64]. In light:dark cycles however growth occurs at shortly before dawn [65]. The gating of growth to this time of day requires two related proteins, PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PIF5; expression of both the *PIF4* and *PIF5* genes is rhythmic and induced by the clock transcription factor CCA1. Expression of *PIF4* and *PIF5* is repressed by the clock during the early night and the proteins themselves are degraded by light hence there is a narrow window before dawn when growth can occur [65]. This is thus an external coincidence pattern since growth depends upon the coincidence of an internal factor (PIF4 and PIF5 proteins) with an external factor, the dark portion of an LD cycle.

Another way that the clock coordinates plant growth is by regulating expression of plant hormones. The plant hormone auxin is responsible for phototropism and other directional growth responses. Genes involved in auxin signalling were overrepresented among clock regulated genes; moreover half the genes induced by auxin were under clock control. Hence, endogenous auxin signalling is clock regulated and the plant's sensitivity to auxin is under clock control [66]. Genes regulated by other plant hormones, including cytokinin, abscisic acid (ABA), methyl jasmonate, gibberellin (GA), brassinosteroid (BR) and salicylic acid, are also more likely than expected by chance to be under clock control [67]. Such findings account in large part for the pervasive effect of the clock upon plant growth and development.

The role of plant hormones in gating hypocotyl elongation has been examined. As noted above in free-running conditions growth occurs in the subjective evening but around dawn in light:dark cycles. Differential gene expression across a light:dark cycle was observed for genes active in the ABA, GA, BR and auxin pathways with peak expression occurring at dawn, coincident with the time of maximal growth [68]. A *cis*-element (CACATG) was identified that was over-represented in the promoters of genes whose expression peaked at subjective dusk in continuous light but at dawn in LD cycles; this motif was sufficient to confer time of day activity on a *luciferase* reporter and was enriched in phytohormone gene promoters [68].

Thus the circadian system and hormone networks interact. In the case of the phytohormone auxin a direct link has been uncovered between the two, providing an example of how clock control of hormone levels may be achieved. The hypocotyls of *rve1* seedlings were shorter than wild type and, like *CCA1*-ox plants, *RVE1*-ox seedlings grew rapidly in short, non-24 h light dark cycles [65,69]. The phenotype of RVE1-ox plants resembled that of auxin overproducers, being tall in constant light and short in darkness; exogenous application of auxin completely restored hypocotyl elongation in *rve1* seedlings. The transcription factor REVEILLE1 (RVE1) is a member of the same protein family as the core circadian components LHY and CCA1. All members contain a proline-rich region preceded by a single MYB domain. Like CCA1 and LHY, RVE1 bound the EE in gene promoters, and, again like *CCA1* and *LHY,* expression of *RVE1* peaked in the morning [69]. However, unlike these genes, *rve1* mutants had no apparent change to the circadian phenotype, although overexpression of *RVE1* did abolish rhythmicity of core clock gene expression [69]. Although exogenous auxin completely restored the hypocotyl elongation response of *rve1*, it did not do so for *lhy* or *cca1;lhy*; moreover, levels of *PIF4* and *PIF5* were normal in *rve1;rve2;rve7* seedlings implying that a different molecular mechanism, that does not involve PIF4 and PIF5, was responsible.

In fact an auxin biosynthesis gene, *YUCCA8* (*YUC8*), was upregulated in *RVE1*-ox seedlings, especially in root tips, an area where auxin is synthesised. The EE is found in the *YUC8* promoter and *YUC8* expression is increased in *RVE*-ox plants [69]. This provides a direct link between the clock and auxin level via the clock-regulated transcription factor *RVE1*.

Given the clock's role in coordinating a plant with its environment, and in coordinating metabolism across the day, it is reasonable to expect that a clock mismatched with the environment will have detrimental effect upon a plant's fitness. Many clock mutants have alterations to flowering time, with some being early flowering due to an inability to perceive daylength and others late flowering due to downregulation of genes in the photoperiodic pathway. An elegant series of experiments with clock mutants of the cyanobacterium *Synechococcus* [70] demonstrated that a clock that resonated with the environment did indeed confer fitness advantages. The interest was strengthened by the observation that photoperiodic flowering could be restored in certain clock mutants of *Arabidopsis* by growing them in a light:dark cycle that matched their endogenous period. For instance, the short period mutant *toc1* is constitutively early flowering in both long and short daylengths in a 24 h light:dark cycle but can distinguish long days from short in cycles where light and dark total 21 h [71].

There have been various studies in *Arabidopsis* addressing the problem of whether a clock matched to the environment improves fitness. Direct evidence for differential success in reproduction is rare, more normally other endpoints such as growth rates or chlorophyll content are used as proxies. Arrhythmic *CCA1*-ox and *LHY*-ox plants showed lower survival rates at short photoperiods, as did the conditionally arrhythmic *elf3* mutant [72]. A later study found that when the environment was matched to the clock's endogenous period, plants produced higher biomass and contained more chlorophyll than in mismatched cycles. CO_2 fixation was also improved in resonating cycles [73]. Both short and long period mutants did better in cycles matched to their endogenous periods than in 24 h cycles, indicating that resonance of the clock is necessary to maintain the proper phase relationship of physiology with the environment. Very recently, hybrid vigour observed in F1 crosses between *A. thaliana* accessions and also in stable allotetraploids between *A. thaliana* and *Arabidopsis arenosa* has been explained with reference to the pattern of *CCA1* and *LHY* expression [74]. Both these clock transcription factors were down regulated in allotetraploids with a concomitant increase in transcripts containing the EE or CBS in their promoters, not only affecting the clock genes *TOC1* and *GI* but also the chlorophyll biosynthesis genes *PROTOCHLOROPHYLLIDE REDUCTASE A* and B (*PORA*, *PORB*). Likely as a direct result of this, chlorophyll levels were high in the allotetraploid plants. Moreover, the involvement of the clock in regulating starch metabolism has already been noted, and many of the genes in this pathway contain the EE or CBS [33]. Such genes were upregulated in the allotetraploids, which accumulated more starch probably as a direct result. In line with this argument, the *CCA1-ox* genotype was noted to accumulate less biomass than the wild type [73] and to have lower levels of chlorophyll [74].

3.3. Photoperiodism and the control of flowering

One of the most visible impacts of the plant clock is photoperiodism, the triggering of events like flowering and senescence in response to the changing seasons. Although temperature plays a role in regulation of such events, for many plants the major trigger is the changing daylength. The clock provides an ideal timing mechanism by which to measure daylength. A daylength sensitive plant can detect whether it is still receiving daylight a given number of hours after dawn. As summer approaches and this condition is met, a "long day plant" will be induced to flower. Alternatively as daylength shortens through autumn, a "short day plant" will be induced to flower or a deciduous tree will be induced to become dormant. The mechanism of daylength measurement is termed the external coincidence model – it relies on a coincidence between the presence of an external signal, daylight, and the sensitive phase of the internal circadian rhythm between about 12 and 20 h after dawn [75–77].

The mechanism is most easily explained with reference to the *Arabidopsis* proteins involved, though orthologues of most of these proteins have been found in a wide range of plant species. The clock component, GI, acts to promote transcription of *CONSTANS* (*CO*) in the afternoon [78]. However, the CO protein formed is only stabilised if light is present. In response to incident light, both phytochrome and cryptochrome photoreceptors are able to prevent degradation of CO allowing CO to regulate transcription of floral meristem identity (FMI) genes [79,80]. These FMI genes in turn regulate the transition to flowering within the shoot meristems. In a long day plant, CO acts as a promoter of transcription of FMI genes; in *Arabidopsis*, CO directly activates *LEAFY* (*LFY*), *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF CO* (*SOC1*) [81] causing the apical meristem to switch from production of leaves to production of flowers [81–85]. In short day plants such as rice, CO acts to inhibit transcription of FMI genes; in rice the CO orthologue, Hd1, inhibits transcriptions of the FT orthologue, Hd3, thereby maintaining the plant in a vegetative state [86].

As the *CO* gene is only active late in the day, the plant is only sensitive to light for regulation of flowering at that time. The mechanism of CO stabilisation is not fully understood but it has been demonstrated that dark degradation of CO is via proteasome pathways [79,87]. In *Arabidopsis*, phyA and cry2 are the major photoreceptors, activated in response to red and blue light, respectively, to

regulate CO stability, but a role for cry1 has also been demonstrated [80]. In rice, phyB is the major photoreceptor involved [88].

The situation is a little more complex in that the timing of the period of sensitivity varies slightly with daylength too. In *Arabidopsis* FLAVIN BINDING KELCH REPEAT F-BOX PROTIN 1 (FKF1) regulates *CO* transcription by degrading a repressor of the *CO* gene called CYCLING DOF FACTOR 1 (CDF1). FKF1 is a protein closely related to ZTL and, like ZTL, binds a flavin chromophore. FKF1 is a blue light photoreceptor whose peak time of mRNA varies with daylength such that the peak protein abundance occurs during the day in long days and the night in short days. Activation by blue light has been proposed to promote binding of FKF1 to CDF1 and therefore promote CDF1 degradation. *FKF1* expression is itself controlled by the clock, peaking in the evening, and the fact that it is also light activated means that FKF1 enhances CO expression specifically in long days when light will still be present at this time of its peak expression [89].

FT forms a key component of this system in that it is responsible for the long distance transmission of the floral stimulus from the site of perception in the leaves to the apical meristem. FT protein, once produced in response to inductive conditions, is transported via the phloem to the apical meristem where it controls the transition to flowering [90,91]. This photoperiodic pathway is summarised in Fig. 2. It should be noted however, that in addition to playing a role in the induction of *FT* via CO, FKF1 may also activate *FT* transcription directly. Mathematical simulation of *FT* induction in *fkf1* mutants failed to reproduce the expression pattern of *FT* seen in real data unless this additional role for FKF1 was invoked [92]. This prediction is an example of the power of modelling-based approaches to the dissection of biological networks.

Not all species employ this mechanism, however. A variation was recently discovered in the classical model short day plant, *Pharbitis* (*Ipomea nil*; formerly, *Pharbitis nil*). As in rice, expression of two *Pharbitis FT* (two orthologues, named *PnFT1* and *2*), is promoted in short days. However, *PnFT* expression does not directly follow *Pn CO* expression. Instead, it is possible to see a divergence in the expression pattern of the two genes. Furthermore, *PnFT* expression is regulated by the circadian clock with a rhythm set by dusk. In *Arabidopsis* and rice the timing of *FT* expression is a function of *CO* expression, the timing of which is set by dawn not dusk, suggesting that *PnFT* may be regulated by a different mechanism from that found in *Arabidopsis* and rice. *PnFT* expression only rises when the night length exceeds 11 h and expression peaks during the subsequent morning and dramatic reduction in expression is observed if the plants receive even a brief night break (a pulse of light during the night) [93]. This latter finding is, therefore, perfectly consistent with the effectiveness of such treatments in suppressing flowering in *Pharbitis* [75]. Hayama et al. (2007), also comment upon the fact that, in tomato, a divergence in the expression pattern of the CO and FT orthologues has also been observed suggesting that an alternative mechanism of FT regulation also exists here too [94,95]. The exact mechanism of FT regulation in these examples has yet to be determined; however this evokes comparisons with the observation of distinct dawn and dusk oscillators found together in *Arabidopsis* [6].

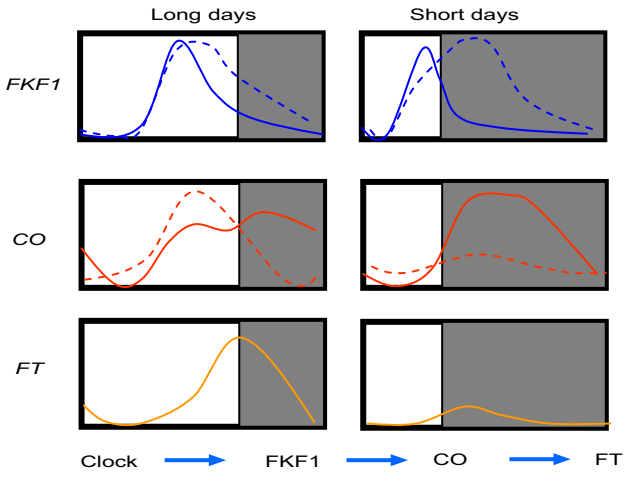

Fig. 2. Photoperiodic induction of flowering. This mechanism can be viewed as an external coincidence model (light coincides with CO protein) or internal coincidence (light coincident with FKF1 ensures daytime peak of CO). Either way, FT expression is induced in long days to trigger the transition to flowering. Solid line: RNA; dotted line: protein; white box: light; grey box: dark. Redrawn from original data in Refs. [89,125].

Deficiency in any of the components of the external coincidence pathway results in a reduced sensitivity to flowering: in long day plants flowering is delayed irrespective of the daylength while in short day plants, flowering is promoted. The practical application of this knowledge has been clearly demonstrated in rice, where increased daylength during the growing season at higher latitudes, limits the growing range of rice to equatorial regions. Over the thousands of years during which rice has been cultivated, farmers have selected lines which could grow over a greater range and it has recently been discovered that several of these strains contain mutations affecting Hd1, Hd3 or phyB [96].

This mechanism may also regulate seasonal events in the life histories of deciduous trees such as the entry to winter dormancy. Aspen trees (*Populus* sp.) overexpressing PHYA neither enter dormancy nor downregulate *FT* when exposed to short days [97] and levels of *CO* also remained high. In addition, the peak time of both *CO* and *FT* varied in different clones growing along a north–south geographical cline and correlated with the critical daylength for growth cessation, implying that in this species dormancy is regulated by the PHYA-FT-CO pathway. However, exposure to low temperature appears to abolish transcriptional rhythms, even in light-grown plants, almost at once [98,99]. However, it is reasonable to suppose that some form of clock remains under these conditions, if only because deciduous trees such as chestnut break dormancy in response to photoperiodic cues so some photoperiodic time-measuring system must continue to run in such a case.

Very recently, a non-transcriptional oscillator has been revealed to operate in darkness in the marine picoalga *Ostreococcus tauri* [100], although this organism also shows circadian regulation of clock components such as *TOC1* [101] and has a transcription–translation feedback loop clock that is a simplified version of that known in *Arabidopsis* [102]. The authors speculate [100] that the non-transcriptional oscillator was the original time-keeping mechanism in the cell and transcriptional rhythms arose later, the two clocks now being coupled together. Whether this is so, and so too in other plants, remains to be determined.

3.4. Photoperiodism reveals the need for complex clocks

The level of complexity in the plant clockwork presents several questions, not least of which is, *Why is the clock so complicated?* For purposes of time measurement a simple hourglass or water clock, that could be reset once per day, would suffice to count out the hours. This question can in part be answered in abstract terms by considering what a clock is for, and in practical ones by observing the behaviour of mutants in which the clock is compromised.

Although circadian clocks continue to run in constant conditions in the laboratory this is not an accurate representation of the conditions in which they have evolved. In the real world, the sun rises, the sun sets, the days grow long and short and a plant, a sessile, photosynthetic organism, must respond and adapt to these changing conditions, coordinating its life cycle with the seasons. All the advantages of coordination of metabolic processes and

constraint of environmental responses to certain times of day will only be gained if the clock is set to the right time. This is the proper purpose of the clock. For this reason, plant clocks, like those in all other organisms, are very sensitive to dawn/dusk signals in order to keep in time with the cycles of the world in which they live. This resetting of the clock, to match internal with external time, is entrainment.

Whether the clock in an organism runs slightly slow or slightly fast, it maintains its synchrony with the day/night cycle by being reset slightly on a daily basis. Both light and temperature can reset the plant clock, though light is by far the more dominant *zeitgeber* (timegiver). Many of the components of the plant clock are sensitive to light, showing light-responsive transcription and/or light-regulation of protein levels and it is these characteristics which allow clock resetting to occur.

In the simplest scenario for resetting the clock, transcription of the dawn-phased central clock components, *CCA1* and *LHY* show strong responses to light [34,103]. The effect of light at dawn is to induce an acute spike in transcript levels. If the clock-driven timing of their transcription is slightly earlier or slightly later than dawn, this acute response will effectively shift their peak expression to coincide with dawn and the clock loop will continue from that new peak time. Indeed, a chemically-induced spike in *CCA1* transcription has been shown to be sufficient to reset the clock [104].

However, a single clock loop with components responsive to dawn and dusk could not track daylength. It would simply be an hourglass reset by the most recent dawn or dusk transition. Plant clock outputs show timing based on information given by both dawn and dusk cues, as sensitivity to the relative times of dawn and dusk also allows the plant to adjust to different daylengths. For example, peak expression of *CAB2* is later in long days than in short days [105]. Therefore, although the *CAB2* gene is known to be a direct target of the CCA1 transcription factor, and peak *CCA1* expression is at dawn, the time of *CAB2* expression cannot be simply set by the time elapsed since peak *CCA1* expression. Instead, an effect of the previous dusk must also be inferred. A number of central clock components are, indeed, sensitive to dusk. The degradation of TOC1 protein following interaction with ZTL is dark-dependent [45], hence TOC1 levels provide information on the time of nightfall.

The explanation for the existence of multiple clock loops in plants lies in this need to track both dawn and dusk. The ''morning loop'', containing *CCA1*, *LHY* and the *PRR* genes, tracks dawn, while the ''evening loop'', containing the *TOC1* and *GI* genes, tracks dusk. The two loops are linked by the fact that both contain CCA1, LHY and TOC1 [106]. Thus the clock is complicated to give it the flexibility to fulfil its purpose. The importance to the plant of such complexity is revealed by the *early flowering 4* (*elf4*) mutant. This retains rhythmic patterns of gene expression in light:dark cycles but these stop after about a day and a half in a constant environment [56,107]. The clock stops in the evening phase, with low *CCA1* and *LHY* and high *TOC1* levels. In layman's terms, the *elf4* clock is no more than an hourglass reset by dawn and dusk. However, the effect on the plant of this damped oscillator is considerable. The *elf4* plant is constitutively early flowering as it has lost the ability to entrain to a photoperiod and hence cannot distinguish between long and short days.

4. Non-photic influences on the plant clock

4.1. Temperature

Circadian clocks are sensitive to temperature changes and a temperature cycle can entrain the plant clock. Although the light:dark cycle is the major stimulus that entrains the clock, plants can also entrain to temperature cycles. The warm period is considered 'day' and the cold period 'night'. Differences of as little as two degrees Celsius can entrain the clock although larger steps produce rhythms with stronger amplitude. The precise mechanism by which the plant clock entrains to temperature is still unclear although it appears to require *EARLY FLOWERING 3* (*ELF3*) as *elf3* null mutants cannot entrain to temperature cycles [48,108]. PRR7 and PRR9 are also required for clock entrainment to temperature cycles [109] as *prr7;prr9* double mutants do not reset their clocks in response to cold pulses. Expression of *PRR7*, *PRR9* and *GI* is elevated during the night in an *elf3* mutant indicating that the normal repression of these genes at night does not occur in this mutant; the response to high temperature pulses was altered in *elf3*. Originally, ELF3 was considered to 'gate' light input to the clock and thus maintain rhythmicity in constant conditions [48]. More recent work has extended this role to gating temperature signals as well [108]. This suggests that ELF3 is essential for rhythmicity under both light and temperature regimes and perhaps is part of the core oscillator. It also implies that pathways are shared between light and temperature entrainment and hence the mechanism may in fact be common to both. However, there is some evidence that there is a second oscillator preferentially entrainable to temperature [21].

Since the purpose of a clock is to keep the correct time it needs to be buffered against the effects of sudden changes in ambient temperature. The rate of a biochemical reaction usually doubles with a 10 °C increase in temperature. A clock whose speed increased in such a way would be useless as a timekeeper and in fact plant clocks are buffered to a very great extent against temperature change ('temperature compensation') with the period remaining relatively unchanged across a fairly wide range of temperature (12–27 °C) [110,111]. Quantitative trait loci (QTL) affecting temperature compensation have been identified in mapping populations of *Arabidopsis*; however the genes with underlying responsibility for this trait do not appear to be the central clock proteins, instead they are regulatory proteins such as GIGANTEA (GI) which alter the speed of the clock [110]. A tentative mechanism for temperature compensation in *Arabidopsis* suggests that amplitude of *GI* rhythms, together with *CCA1* and *LHY*, differ across the range of temperature, and this dynamic balance maintains a stable period [10].

However, the clock appears to stop at low temperature in deciduous trees such as sweet chestnut (*Castanea sativa*) in winter [98]: when the trees are dormant, cycling of the clock components *CCA1* and *LHY* ceases, and the same response occurs if they are chilled to 4 °C; rhythmicity is restored when the seedlings are returned to 22 °C. More recently, similar effects of chilling have been observed in *Arabidopsis.* In light:dark cycles at 4 °C rhythmic gene expression continues but the amplitude of expression is strongly damped; however, in constant light at the same temperature rhythmicity of clock-controlled genes is abolished within 24 h [99,112]. This result strongly suggests that there are limits to temperature-compensation in plants. It is clear that chilling to 4 °C is not lethal either to chestnut trees or to *Arabidopsis*; *Arabidopsis* plants continue to grow and will eventually set seed, although growth is slower and seedset delayed under these conditions. What exposure to low temperature does do is induce expression of a suite of cold-responsive genes [113,114] that protect the plant from chilling damage.

4.2. Sugar

Transcriptional studies with whole genome microarrays have revealed that a large proportion of the *Arabidopsis* genome is expressed rhythmically (up to 89% of expressed genes are rhythmic

under one condition or another [58]). In constant light, peak expression of genes occurs at all phases of the 24 h cycle (see for instance [33] or [66]) whereas in entrained conditions the peak phase may alter with the zeitgeber [58]. Analysis of temporal gene clusters reveals co-ordinated expression of genes with related function, for example phenylpropanoid biosynthesis genes peak before subjective dawn whilst photosynthesis genes do so during the middle of the subjective day and starch mobilising enzymes during the night. The temporal partition of physiology, especially aspects such as sugar production and utilisation, implies a major role of the clock is to co-ordinate aspects of plant metabolism.

Plants cannot be grown in the dark for extended periods since they are autotrophic, deriving their energy from photosynthesis. Extending the period of darkness beyond that of the expected night leads to carbon depletion and changes expression of hundreds of genes regulating carbohydrate metabolism [115,116]; the appearance of plants also changes as they become rapidly aetiolated and yellowed; death through starvation will follow if light is not restored. However, if sucrose is supplied to provide a carbon source, plants will survive long periods of growth in the dark. Moreover, sugar increases the brightness of the *LUCIFERASE* reporter, often used to study patterns of clock gene expression [117] and also reduces the amount of variation in period lengths observed between individuals of a population [118]. For these reasons, many plant clock laboratories routinely grow seedlings on media containing 2–3% sucrose, continuing to do so even if *LUCIFERASE* activity is not being observed or if the environmental regime consists of constant light or light:dark cycles. However, the addition of sugar presents a potential problem for researchers and one that perhaps has not been adequately appreciated. In fact, the majority of the studies which have contributed to the current model of the plant clock derive entirely from investigations of young seedlings (<14 days post germination) grown on sterile plates of a defined medium in the presence of sucrose.

Sucrose does not present an osmotic stress to seedlings [118] but it does delay germination [119] and inhibit hypocotyl elongation, apparently by interfering with phytochrome A signalling [120]. Studies in which plants are grown on soil for longer periods have revealed the extent to which plant metabolism is geared to controlling sugar production and starch mobilisation. A study on older plants revealed starch mobilisation is indeed very precisely timed to the expected night length [121]; when the night was unexpectedly extended plants 'ran out' of resources resulting in a temporary repression of genes required for biosynthesis and growth. Moreover, many sugar-responsive genes showed large variations in expression across a light:dark cycle matched to the variation in sugar levels and this study revealed that sugar modified the expression of half the clock-regulated genes. In fact sugar (i.e., the product of photosynthesis) rather than light itself made a greater contribution to the regulation of gene expression during a light:dark cycle [122]. However, about half the genes identified as being regulated by the clock were expressed in the same manner in a mutant that cannot accumulate starch (*phosphoglucomutase; pgm*) as in wild type plants. Since sugars are exhausted 6–8 h earlier in *pgm* plants, this implies that the clock itself is buffered against the effects of sugar depletion [122].

There have been relatively few studies directly addressing the effects of exogenous sugars on the plant clock. Sugar may be involved in regulating either the clock itself or its outputs, as the free-running period of plants shortens when they are grown in the presence of sucrose although, surprisingly, clock gene expression is very little altered [118]. This may involve cross talk with temperature signalling pathways as the *sensitive to freezing 6* (*sfr6*) mutant, which expresses cold responsive genes only to a low level, does not show this response. However *sfr6* is not globally insensitive to sugar as other responses to sucrose are retained.

The effect of sugar on clock period suggests a mechanism to explain the observation known as "Aschoff's rule", which states that day-active organisms have shorter circadian periods in brighter light [37]. In plants, both increasing the light level and supplementation with sucrose causes shortening of the free-running period [39,118]. Since plants are photosynthetic, the shortening of the circadian period due to the effect of sugar may be because, at a cellular level, sugar produces an effect resembling that of an increase in light levels (which causes an increase in photosynthetic activity and hence of sugars).

There is also evidence that sugar can also moderate entrainment of the plant clock. Rhythmic gene expression is observed in the roots of plants grown in light:dark cycles but not in constant light even though in both cases the roots themselves are in darkness [36]. In fact, this study showed that many of the genes involved in the feedback loops considered to comprise the clock did not oscillate in roots of five-week old plants, including *GI, LUX, PRR3, PRR5, ELF3, ELF4* and *TOC1* itself, although both *CCA1* and *LHY* expression remained rhythmic in roots. Such results suggest that the free-running root clock is a pared down version of the three-loop clock predicted from studying light-grown seedlings, thus extending the observation that many clock controlled outputs cease to be rhythmic in the absence of environmental input.

5. One clock or many?

It is common to discuss the plant clock as if it were a single entity composed of the interlocking transcription–translation feedback loops (TTFL) discussed in the preceding sections. Whether it is in fact so is not as clear perhaps as researchers would like to think it. Even aerial tissues however may contain more than one clock. The *Arabidopsis* cotyledon appears to contain two oscillators, one (of which the behaviour of *CAB2* is a 'hand') entrains preferentially to light cycles and the other, (indicated by *CATALASE3*) to temperature cycles [21]. These oscillators had different free-running periods from each other, indicating that they are generated by different clocks. Similarly, the free-running periods of phyB and *CAB2* rhythms differed from each other in *Arabidopsis* seedlings [123]. In this latter case, although the free-running periods differed, mutations affected each in parallel suggesting that the clocks generating these rhythms share common components but are modified in a tissue-specific manner, and that clocks entrain independently.

The issue of if and how temporal information is transmitted between plant organs is another area of debate with some studies showing tissue autonomy (e.g., [124], where two cotyledons entrained and subsequently free-ran in opposite phase to each other) and others tissue dependence (e.g., [36] where rhythms in roots were entrained by a signal from the shoot, at least in light:dark cycles). This latter study also presented evidence that the three loop oscillator may not run in all tissues in all circumstances; if this is the case, it would suggest that light might provide an important positive-acting factor, maintaining expression levels of clock genes, against which periodic negative feedback acts to drive a rhythm. This would create a distinction between the central clock loops that operate in above ground tissue and those which operate in roots. In roots only *CCA1*, *LHY*, *PRR7*, and *PRR9* expression behaved rhythmically in constant conditions [36]. Expression of *TOC1* and *GI* ceased to oscillate. Consistent with this, in roots CCA1 and LHY are unable to mediate inhibition of transcription of genes containing the evening element, including *TOC1* [36]. Essentially, this means that a circadian rhythm can be maintained by the "morning loop" of genes alone in root tissues without the requirement for the "evening" TOC1 loop.

This raises a question as to the reason for the involvement of TOC1 in aerial tissues. It is tempting to speculate that the TOC1 loop in aerial tissues is solely present to allow adaptation to day-

length. TOC1 forms part of the dusk-tracking loop [6] and it is, perhaps, no surprise that in roots there is no longer a co-occurrence of both dawn and dusk-tracking loops. In roots, a functional circadian clock could still provide a significant advantage. However, in the absence of photosynthetic processes, daylength tracking could be perceived as being significantly less important.

6. Conclusions

The circadian system is an integral part of plant biology, regulating most aspects of daily and seasonal behaviour. In this review we have attempted to provide an overview of the plant clock, drawing attention to its plastic nature and adaptability which allow plants to respond flexibly to changing conditions. Many unanswered questions remain, not least how the clock is influenced by non-photic cues such as sugar level and the extent to which it keeps running at low temperatures or in constant conditions. The nature of clocks in different tissues and the levels of circadian communication between plant organs also remain imperfectly understood.

References

[1] Hansard (1880). Statutes (Definition of Time) 3rd Reading in: Minutes of the House of Commons, Vol. 8th July 1880, 253 Her Majesty's Stationery Office.
[2] Ripperger, J.A., Jud, C. and Albrecht, U. (2011) The daily rhythm of mice. FEBS Lett. 585, 1384–1392.
[3] Schafmeier, T. and Diernfellner, A.C.R. (2011) Light input and processing in the circadian clock of *Neurospora*. FEBS Lett. 585, 1467–1473.
[4] Peschel, N. and Helfrich-Förster, C. (2011) Setting the clock – by nature: circadian rhythms in the fruitfly *Drosophila melanogaster*. FEBS Lett. 585, 1435–1442.
[5] Locke, J.C., Millar, A.J. and Turner, M.S. (2005) Modelling genetic networks with noisy and varied experimental data: the circadian clock in *Arabidopsis thaliana*. J. Theor. Biol. 234, 383–393.
[6] Locke, J.C. et al. (2006) Experimental validation of a predicted feedback loop in the multi-oscillator clock of *Arabidopsis thaliana*. Mol. Syst. Biol. 2, 59.
[7] Zeilinger, M.N., Farre, E.M., Taylor, S.R., Kay, S.A. and Doyle 3rd, F.J. (2006) A novel computational model of the circadian clock in *Arabidopsis* that incorporates PRR7 and PRR9. Mol. Syst. Biol. 2, 58.
[8] Millar, A.J. (2004) Input signals to the plant circadian clock. J. Exp. Bot. 55, 277–283.
[9] Franklin, K.A., Larner, V.S. and Whitelam, G.C. (2005) The signal transducing photoreceptors of plants. Int. J. Dev. Biol. 49, 653–664.
[10] Gould, P.D. et al. (2006) The molecular basis of temperature compensation in the *Arabidopsis* circadian clock. Plant Cell 18, 1177–1187.
[11] Alabadi, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Mas, P. and Kay, S.A. (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. Science 293, 880–883.
[12] Perales, M. and Mas, P. (2007) A functional link between rhythmic changes in chromatin structure and the *Arabidopsis* biological clock. Plant Cell 19, 2111–2123.
[13] Matsushika, A., Makino, S., Kojima, M. and Mizuno, T. (2000) Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana*: insight into the plant circadian clock. Plant Cell Physiol. 41, 1002–1012.
[14] Makino, S., Matsushika, A., Kojima, M., Oda, Y. and Mizuno, T. (2001) Light response of the circadian waves of the APRR1/TOC1 quintet: when does the quintet start singing rhythmically in *Arabidopsis*? Plant Cell Physiol. 42, 334–339.
[15] Nakamichi, N., Matsushika, A., Yamashino, T. and Mizuno, T. (2003) Cell autonomous circadian waves of the APRR1/TOC1 quintet in an established cell line of *Arabidopsis thaliana*. Plant Cell Physiol. 44, 360–365.
[16] Mas, P. (2008) Circadian clock function in *Arabidopsis thaliana*: time beyond transcription. Trends Cell Biol. 18, 273–281.
[17] Pruneda-Paz, J.L., Breton, G., Para, A. and Kay, S.A. (2009) A functional genomics approach reveals CHE as a component of the *Arabidopsis* circadian clock. Science 323, 1481–1485.
[18] Eriksson, M.E., Hanano, S., Southern, M.M., Hall, A. and Millar, A.J. (2003) Response regulator homologues have complementary, light-dependent functions in the *Arabidopsis* circadian clock. Planta 218, 159–162.
[19] Ito, S., Matsushika, A., Yamada, H., Sato, S., Kato, T., Tabata, S., Yamashino, T. and Mizuno, T. (2003) Characterization of the APRR9 pseudo-response regulator belonging to the APRR1/TOC1 quintet in *Arabidopsis thaliana*. Plant Cell Physiol. 44, 1237–1245.
[20] Yamamoto, Y. et al. (2003) Comparative genetic studies on the APRR5 and APRR7 genes belonging to the APRR1/TOC1 quintet implicated in circadian rhythm, control of flowering time, and early photomorphogenesis. Plant Cell Physiol. 44, 1119–1130.
[21] Michael, T.P., Salome, P.A. and McClung, C.R. (2003) Two *Arabidopsis* circadian oscillators can be distinguished by differential temperature sensitivity. Proc. Natl. Acad. Sci. USA 100, 6878–6883.
[22] Farre, E.M., Harmer, S.L., Harmon, F.G., Yanovsky, M.J. and Kay, S.A. (2005) Overlapping and distinct roles of PRR7 and PRR9 in the *Arabidopsis* circadian clock. Curr. Biol. 15, 47–54.
[23] Fowler, S., Lee, K., Onouchi, H., Samach, A., Richardson, K., Morris, B., Coupland, G. and Putterill, J. (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. EMBO J. 18, 4679–4688.
[24] Martin-Tryon, E.L., Kreps, J.A. and Harmer, S.L. (2007) GIGANTEA acts in blue light signaling and has biochemically separable roles in circadian clock and flowering time regulation. Plant Physiol. 143, 473–486.
[25] Nakamichi, N., Kita, M., Ito, S., Yamashino, T. and Mizuno, T. (2005) Pseudo-response regulators, PRR9, PRR7, and PRR5, play together essential roles close to the circadian clock of *Arabidopsis thaliana*. Plant Cell Physiol.
[26] Ding, Z. and Davis, S.J. (2007) A complex genetic interaction between *Arabidopsis thaliana* TOC1 and CCA1/LHY in driving the circadian clock and in output regulation. Genetics.
[27] Hogenesch, J.B. and Herzog, E.D. (2011) Intracellular and intercellular processes determine robustness of the circadian clock. FEBS Lett. 585, 1427–1434.
[28] Han, L., Mason, M., Risseeuw, E.P., Crosby, W.L. and Somers, D.E. (2004) Formation of an SCF(ZTL) complex is required for proper regulation of circadian timing. Plant J. 40, 291–301.
[29] Kiba, T., Henriques, R., Sakakibara, H. and Chua, N.H. (2007) Targeted degradation of PSEUDO-RESPONSE REGULATOR5 by an SCFZTL complex regulates clock function and photomorphogenesis in *Arabidopsis thaliana*. Plant Cell 19, 2516–2530.
[30] Kim, W.Y. et al. (2007) ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature 449, 356–360.
[31] Para, A., Farre, E.M., Imaizumi, T., Pruneda-Paz, J.L., Harmon, F.G. and Kay, S.A. (2007) PRR3 Is a vascular regulator of TOC1 stability in the *Arabidopsis* circadian clock. Plant Cell 19, 3462–3473.
[32] Fujiwara, S., Wang, L., Han, L., Suh, S.S., Salome, P.A., McClung, C.R. and Somers, D.E. (2008) Post-translational regulation of the *Arabidopsis* circadian clock through selective proteolysis and phosphorylation of pseudo-response regulator proteins. J. Biol. Chem. 283, 23073–23083.
[33] Harmer, S.L. et al. (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science 290, 2110–2113.
[34] Wang, Z.Y. and Tobin, E.M. (1998) Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. Cell 93, 1207–1217.
[35] Michael, T.P. and McClung, C.R. (2003) Enhancer trapping reveals widespread circadian clock transcriptional control in *Arabidopsis*. Plant Physiol. 132, 629–639.
[36] James, A.B., Monreal, J.A., Nimmo, G.A., Kelly, C.L., Herzyk, P., Jenkins, G.I. and Nimmo, H.G. (2008) The circadian clock in *Arabidopsis* roots is a simplified slave version of the clock in shoots. Science 322, 1832–1835.
[37] Aschoff, J. (1979) Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. Z. Tierpsychol. 49, 225–249.
[38] Devlin, P.F., Robson, P.R., Patel, S.R., Goosey, L., Sharrock, R.A. and Whitelam, G.C. (1999) Phytochrome D acts in the shade-avoidance syndrome in *Arabidopsis* by controlling elongation growth and flowering time. Plant Physiol. 119, 909–915.
[39] Devlin, P.F. and Kay, S.A. (2000) Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. Plant Cell 12, 2499–2510.
[40] Somers, D.E., Devlin, P.F. and Kay, S.A. (1998) Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. Science 282, 1488–1490.
[41] Martinez-Garcia, J.F., Huq, E. and Quail, P.H. (2000) Direct targeting of light signals to a promoter element-bound transcription factor. Science 288, 859–863.
[42] Bauer, D. et al. (2004) Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in *Arabidopsis*. Plant Cell 16, 1433–1445.
[43] Viczian, A., Kircher, S., Fejes, E., Millar, A.J., Schafer, E., Kozma-Bognar, L. and Nagy, F. (2005) Functional characterisation of phytochrome interacting factor 3 for the *Arabidopsis thaliana* circadian clockwork. Plant Cell Physiol.
[44] Lee, J. et al. (2007) Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell 19, 731–749.
[45] Mas, P., Kim, W.Y., Somers, D.E. and Kay, S.A. (2003) Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. Nature 426, 567–570.
[46] Somers, D.E., Schultz, T.F., Milnamow, M. and Kay, S.A. (2000) ZEITLUPE encodes a novel clock-associated PAS protein from *Arabidopsis*. Cell 101, 319–329.
[47] Toth, R., Kevei, E., Hall, A., Millar, A.J., Nagy, F. and Kozma-Bognar, L. (2001) Circadian clock-regulated expression of phytochrome and cryptochrome genes in *Arabidopsis*. Plant Physiol. 127, 1607–1616.

[48] McWatters, H.G., Bastow, R.M., Hall, A. and Millar, A.J. (2000) The ELF3 zeitnehmer regulates light signalling to the circadian clock. Nature 408, 716–720.
[49] Covington, M.F., Panda, S., Liu, X.L., Strayer, C.A., Wagner, D.R. and Kay, S.A. (2001) ELF3 modulates resetting of the circadian clock in *Arabidopsis*. Plant Cell 13, 1305–1315.
[50] Liu, X.L., Covington, M.F., Fankhauser, C., Chory, J. and Wagner, D.R. (2001) ELF3 encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis* PHYB signal transduction pathway. Plant Cell 13, 1293–1304.
[51] Allen, T., Koustenis, A., Theodorou, G., Somers, D.E., Kay, S.A., Whitelam, G.C. and Devlin, P.F. (2006) *Arabidopsis* FHY3 specifically gates phytochrome signaling to the circadian clock. Plant Cell 18, 2506–2516.
[52] Lin, R., Ding, L., Casola, C., Ripoll, D.R., Feschotte, C. and Wang, H. (2007) Transposase-derived transcription factors regulate light signaling in *Arabidopsis*. Science 318, 1302–1305.
[53] Hall, A. et al. (2003) The TIME FOR COFFEE gene maintains the amplitude and timing of *Arabidopsis* circadian clocks. Plant Cell 15, 2719–2729.
[54] Ding, Z., Millar, A.J., Davis, A.M. and Davis, S.J. (2007) TIME FOR COFFEE encodes a nuclear regulator in the *Arabidopsis thaliana* circadian clock. Plant Cell 19, 1522–1536.
[55] Duc, C., Cellier, F., Lobreaux, S., Briat, J.F. and Gaymard, F. (2009) Regulation of iron homeostasis in *Arabidopsis thaliana* by the clock regulator time for coffee. J. Biol. Chem. 284, 36271–36281.
[56] McWatters, H.G. et al. (2007) ELF4 is required for oscillatory properties of the circadian clock. Plant Physiol. 144, 391–401.
[57] Kikis, E.A., Khanna, R. and Quail, P.H. (2005) ELF4 is a phytochrome-regulated component of a negative-feedback loop involving the central oscillator components CCA1 and LHY. Plant J. 44, 300–313.
[58] Michael, T.P. et al. (2008) Network discovery pipeline elucidates conserved time-of-day-specific cis-regulatory modules. PLoS Genet. 4, e14.
[59] Wang, Z.Y., Kenigsbuch, D., Sun, L., Harel, E., Ong, M.S. and Tobin, E.M. (1997) A Myb-related transcription factor is involved in the phytochrome regulation of an *Arabidopsis* Lhcb gene. Plant Cell 9, 491–507.
[60] Harmer, S.L. and Kay, S.A. (2005) Positive and negative factors confer phase-specific circadian regulation of transcription in *Arabidopsis*. Plant Cell 17, 1926–1940.
[61] Michael, T.P. and McClung, C.R. (2002) Phase-specific circadian clock regulatory elements in *Arabidopsis*. Plant Physiol. 130, 627–638.
[62] Hudson, M.E. and Quail, P.H. (2003) Identification of promoter motifs involved in the network of phytochrome A-regulated gene expression by combined analysis of genomic sequence and microarray data. Plant Physiol. 133, 1605–1616.
[63] Ripperger, J.A. and Schibler, U. (2006) Rhythmic CLOCK-BMAL1 binding to multiple E-box motifs drives circadian Dbp transcription and chromatin transitions. Nat. Genet. 38, 369–374.
[64] Dowson-Day, M.J. and Millar, A.J. (1999) Circadian dysfunction causes aberrant hypocotyl elongation patterns in *Arabidopsis*. Plant J. 17, 63–71.
[65] Nozue, K., Covington, M.F., Duek, P.D., Lorrain, S., Fankhauser, C., Harmer, S.L. and Maloof, J.N. (2007) Rhythmic growth explained by coincidence between internal and external cues. Nature.
[66] Covington, M.F. and Harmer, S.L. (2007) The circadian clock regulates auxin signaling and responses in *Arabidopsis*. PLoS Biol. 5, e222.
[67] Covington, M.F., Maloof, J.N., Straume, M., Kay, S.A. and Harmer, S.L. (2008) Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. Genome Biol. 9, R130.
[68] Michael, T.P., Breton, G., Hazen, S.P., Priest, H., Mockler, T.C., Kay, S.A. and Chory, J. (2008) A morning-specific phytohormone gene expression program underlying rhythmic plant growth. PLoS Biol. 6, e225.
[69] Rawat, R. et al. (2009) REVEILLE1, a Myb-like transcription factor, integrates the circadian clock and auxin pathways. Proc. Natl. Acad. Sci. USA 106, 16883–16888.
[70] Ouyang, Y., Andersson, C.R., Kondo, T., Golden, S.S. and Johnson, C.H. (1998) Resonating circadian clocks enhance fitness in cyanobacteria. Proc. Natl. Acad. Sci. USA 95, 8660–8664.
[71] Strayer, C. et al. (2000) Cloning of the *Arabidopsis* clock gene TOC1, an autoregulatory response regulator homolog. Science 289, 768–771.
[72] Green, R.M., Tingay, S., Wang, Z.Y. and Tobin, E.M. (2002) Circadian rhythms confer a higher level of fitness to *Arabidopsis* plants. Plant Physiol. 129, 576–584.
[73] Dodd, A.N. et al. (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science 309, 630–633.
[74] Ni, Z., Kim, E.D., Ha, M., Lackey, E., Liu, J., Zhang, Y., Sun, Q. and Chen, Z.J. (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature 457, 327–331.
[75] Thomas, B. and Vince-Prue, D. (1997) Photoperiodism in Plants, second ed., Academic Press, London.
[76] Pittendrigh, C.S. and Minis, D.H. (1964) The entrainment of circadian oscillations by light and their role as photoperiodic clocks. Am. Nat. 98, 261–294.
[77] Bunning, E. (1936) Die endogene Tagesrhythmikals Grundlage der photoperiodischen Reaktion. Ber. Dtsch. Bot. Ges. 54, 590–607.
[78] Mizoguchi, T. et al. (2005) Distinct roles of GIGANTEA in promoting flowering and regulating circadian rhythms in *Arabidopsis*. Plant Cell 17, 2255–2270.
[79] Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A. and Coupland, G. (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 303, 1003–1006.
[80] Yanovsky, M.J. and Kay, S.A. (2002) Molecular basis of seasonal time measurement in *Arabidopsis*. Nature 419, 308–312.
[81] Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F. and Coupland, G. (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science 288, 1613–1616.
[82] Borner, R., Kampmann, G., Chandler, J., Gleissner, R., Wisman, E., Apel, K. and Melzer, S. (2000) A MADS domain gene involved in the transition to flowering in *Arabidopsis*. Plant J. 24, 591–599.
[83] Lee, H. et al. (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Genes Dev. 14, 2366–2376.
[84] Kardailsky, I. et al. (1999) Activation tagging of the floral inducer FT. Science 286, 1962–1965.
[85] Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F. and Meyerowitz, E.M. (1992) LEAFY controls floral meristem identity in *Arabidopsis*. Cell 69, 843–859.
[86] Hayama, R. and Coupland, G. (2003) Shedding light on the circadian clock and the photoperiodic control of flowering. Curr. Opin. Plant Biol. 6, 13–19.
[87] Laubinger, S. et al. (2006) *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. Development 133, 3213–3222.
[88] Izawa, T., Oikawa, T., Sugiyama, N., Tanisaka, T., Yano, M. and Shimamoto, K. (2002) Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. Genes Dev. 16, 2006–2020.
[89] Imaizumi, T., Tran, H.G., Swartz, T.E., Briggs, W.R. and Kay, S.A. (2003) FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. Nature 426, 302–306.
[90] Corbesier, L. et al. (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. Science 316, 1030–1033.
[91] Jaeger, K.E. and Wigge, P.A. (2007) FT protein acts as a long-range signal in *Arabidopsis*. Curr. Biol. 17, 1050–1054.
[92] Salazar, J.D. et al. (2009) Prediction of photoperiodic regulators from quantitative gene circuit models. Cell 139, 1170–1179.
[93] Hayama, R., Agashe, B., Luley, E., King, R. and Coupland, G. (2007) A circadian rhythm set by dusk determines the expression of FT homologs and the short-day photoperiodic flowering response in Pharbitis. Plant Cell 19, 2988–3000.
[94] Ben-Naim, O., Eshed, R., Parnis, A., Teper-Bamnolker, P., Shalit, A., Coupland, G., Samach, A. and Lifschitz, E. (2006) The CCAAT binding factor can mediate interactions between CONSTANS-like proteins and DNA. Plant J. 46, 462–476.
[95] Lifschitz, E., Eviatar, T., Rozman, A., Shalit, A., Goldshmidt, A., Amsellem, Z., Alvarez, J.P. and Eshed, Y. (2006) The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proc. Natl. Acad. Sci. USA 103, 6398–6403.
[96] Izawa, T., Konishi, S., Shomura, A. and Yano, M. (2009) DNA changes tell us about rice domestication. Curr. Opin. Plant Biol. 12, 185–192.
[97] Bohlenius, H., Huang, T., Charbonnel-Campaa, L., Brunner, A.M., Jansson, S., Strauss, S.H. and Nilsson, O. (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312, 1040–1043.
[98] Ramos, A., Perez-Solis, E., Ibanez, C., Casado, R., Collada, C., Gomez, L., Aragoncillo, C. and Allona, I. (2005) Winter disruption of the circadian clock in chestnut. Proc. Natl. Acad. Sci. USA 102, 7037–7042.
[99] Bieniawska, Z., Espinoza, C., Schlereth, A., Sulpice, R., Hincha, D.K. and Hannah, M.A. (2008) Disruption of the *Arabidopsis* circadian clock is responsible for extensive variation in the cold-responsive transcriptome. Plant Physiol. 147, 263–279.
[100] O'Neill, J.S., van Ooijen, G., Dixon, L.E., Troein, C., Corellou, F., Bouget, F.-Y., Reddy, A.B. and Millar, A.J. (2011) Circadian rhythms persist without transcription in a eukaryote. Nature 469, 554–558.
[101] Djouani-Tahri el, B., Motta, J.P., Bouget, F.Y. and Corellou, F. (2010) Insights into the regulation of the core clock component TOC1 in the green picoeukaryote Ostreococcus. Plant Signal Behav. 5, 332–335.
[102] Troein, C., Corellou, F., Dixon, L.E., van Ooijen, G., O'Neill, J.S., Bouget, F.-Y. and Millar, A.J. (2011) Multiple light inputs to a simple clock circuit allow complex biological rhythms. Plant J. doi: 10.1111/j.1365-313X.2011.04489.x.
[103] Schaffer, R., Ramsay, N., Samach, A., Corden, S., Putterill, J., Carre, I.A. and Coupland, G. (1998) The late elongated hypocotyl mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. Cell 93, 1219–1229.
[104] Knowles, S.M., Lu, S.X. and Tobin, E.M. (2008) Testing time: can ethanol-induced pulses of proposed oscillator components phase shift rhythms in *Arabidopsis*? J. Biol. Rhythms 23, 463–471.
[105] Darrah, C., Taylor, B.L., Edwards, K.D., Brown, P.E., Hall, A. and McWatters, H.G. (2006) Analysis of phase of LUCIFERASE expression reveals novel circadian quantitative trait loci in *Arabidopsis*. Plant Physiol. 12, 23.
[106] Locke, J.C., Southern, M.M., Kozma-Bognar, L., Hibberd, V., Brown, P.E., Turner, M.S. and Millar, A.J. (2005) Extension of a genetic network model by iterative experimentation and mathematical analysis. Mol. Syst. Biol. 1, 0013.
[107] Doyle, M.R., Davis, S.J., Bastow, R.M., McWatters, H.G., Kozma-Bognar, L., Nagy, F., Millar, A.J. and Amasino, R.M. (2002) The ELF4 gene controls circadian rhythms and flowering time in *Arabidopsis thaliana*. Nature 419, 74–77.
[108] Thines, B. and Harmon, F.G. (2010) Ambient temperature response establishes ELF3 as a required component of the core *Arabidopsis* circadian clock. Proc. Natl. Acad. Sci. USA.
[109] Salome, P.A. and McClung, C.R. (2005) PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the *Arabidopsis* circadian clock. Plant Cell.

[110] Edwards, K.D., Lynn, J.R., Gyula, P., Nagy, F. and Millar, A.J. (2005) Natural allelic variation in the temperature-compensation mechanisms of the *Arabidopsis thaliana* circadian clock. Genetics 170, 387–400.
[111] Edwards, K.D. et al. (2006) FLOWERING LOCUS C mediates natural variation in the high-temperature response of the *Arabidopsis* circadian clock. Plant Cell 18, 639–650.
[112] Espinoza, C., Bieniawska, Z., Hincha, D.K. and Hannah, M.A. (2008) Interactions between the circadian clock and cold-response in *Arabidopsis*. Plant Signal Behav. 3, 593–594.
[113] Gilmour, S.J., Zarka, D.G., Stockinger, E.J., Salazar, M.P., Houghton, J.M. and Thomashow, M.F. (1998) Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. Plant J. 16, 433–442.
[114] Gilmour, S.J., Sebolt, A.M., Salazar, M.P., Everard, J.D. and Thomashow, M.F. (2000) Overexpression of the *Arabidopsis* CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol. 124, 1854–1865.
[115] Contento, A.L., Kim, S.J. and Bassham, D.C. (2004) Transcriptome profiling of the response of *Arabidopsis* suspension culture cells to Suc starvation. Plant Physiol. 135, 2330–2347.
[116] Thimm, O. et al. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. 37, 914–939.
[117] Millar, A.J., Short, S.R., Hiratsuka, K., Chua, N.-H. and Kay, S.A. (1992) Firefly luciferase as a reporter of regulated gene expression in higher plants. Plant Mol. Biol. Rep. 10, 324–337.
[118] Knight, H., Thomson, A.J. and McWatters, H.G. (2008) Sensitive to freezing6 integrates cellular and environmental inputs to the plant circadian clock. Plant Physiol. 148, 293–303.
[119] Dekkers, B.J., Schuurmans, J.A. and Smeekens, S.C. (2004) Glucose delays seed germination in *Arabidopsis* thaliana. Planta 218, 579–588.
[120] Dijkwel, P.P., Huijser, C., Weisbeek, P.J., Chua, N.H. and Smeekens, S.C.M. (1997) Sucrose control of phytochrome A signaling in *Arabidopsis*. Plant Cell 9, 583–595.
[121] Gibon, Y. et al. (2004) Adjustment of diurnal starch turnover to short days: depletion of sugar during the night leads to a temporary inhibition of carbohydrate utilization, accumulation of sugars and post-translational activation of ADP-glucose pyrophosphorylase in the following light period. Plant J. 39, 847–862.
[122] Blasing, O.E. et al. (2005) Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in *Arabidopsis*. Plant Cell 17, 3257–3281.
[123] Hall, A., Kozma-Bognar, L., Bastow, R.M., Nagy, F. and Millar, A.J. (2002) Distinct regulation of CAB and PHYB gene expression by similar circadian clocks. Plant J. 32, 529–537.
[124] Thain, S.C., Hall, A. and Millar, A.J. (2000) Functional independence of circadian clocks that regulate plant gene expression. Curr. Biol. 10, 951–956.
[125] Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F. and Coupland, G. (2001) CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature 410, 1116–1120.